CONJUNCTIVAL BACTERIAL FLORA IN DIABETIC PATIENTS

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Abstract

Conjunctival flora refers to population of microorganisms that dwell within the eyes of healthy individuals and is important in maintaining a healthy ocular surface and normal conjunctival function. Conjunctival flora may be altered by a variety of factors that include age, immunosuppression and geography. Immune function is compromised in diabetes mellitus. The aim of the present study was to see the pattern of conjunctival bacterial flora in diabetic and non-diabetic patients. This cross sectional study was carried out in BSMMU during the period of January 2011 to December 2011. Total 500 conjunctival swabs were collected from both eyes of 50 diabetic patients attending OPD of Endocrinology Department of BSMMU and 200 non-diabetic individuals. Significant number of culture was positive in diabetic patients (64.0%) compared to that of non-diabetic individuals (38.0%). *Staphylococcus epidermidis* was predominant in both study groups (diabetic vs non-diabetic: 41.3% vs 65.26%). *Staphylococcus aureus* (15.22%), *Escherichia coli* (6.52%) and *Enterobacter* (8.33%) were isolated in diabetic patients. Rate of positive culture in both and single eyes were higher in diabetic (28%, 36.0%) than that of non-diabetic individuals (9.5%, 28.5%).

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Key words: Conjunctival flora, diabetes mellitus (DM).

Introduction

The conjunctiva is a thin, transparent, smooth and humid membrane that covers and protects the bulbar and the palpebral conjunctiva.[1] The conjunctiva is kept moist and healthy by tear which contains lysozyme, IgA, IgG, â-lysine, lactoferrin, complement and multiple antibacterial enzymes.[2]

In a healthy person, surface tissues such as skin and mucous membranes are constantly in contact with environmental organisms and becomes colonized by various micro-organisms which are referred to as normal flora.[3] Bacteria and fungi are considered as normal flora of conjunctiva whereas viruses and parasites are not considered as the members of the normal flora.[4]

The predominant microorganisms of conjunctiva are *Staphylococcus epidermidis* (30-80%), *Diphtheroids* (5-83%), *Micrococcus sp.* (1-28%) and *Staphylococcus aureus* (3-25%).[5] In addition, *Streptococcus pyogenes* (0-3%), *Streptococcus pneumoniae* (0-3%), *Streptococcus viridans* (0-1%), *Moraxella catarrhalis* (2-5%), *Haemophilus influenzae* (0-1%), *Klebsiella sp.* (0-0.5%), *Escherichia coli* (0-1%) and *Pseudomonas aeruginosa* (0-2%) are occasionally found.

Normal conjunctival flora remains relatively consistent among human populations. However, it may be altered by a variety of factors including age, immunosuppression, ocular inflammation, dry eye, use of contact lens use, antimicrobials, surgery, external exposure, climate and geography. Some members of the conjunctival flora play a pathogenic role in diabetes mellitus when immune function is compromised, which may lead to serious infection.[6]

Diabetic patients are prone to develop infection including eye infections. Common eye infections in diabetics are blepharitis, conjunctivitis, keratitis, stye, chalazion and orbital cellulitis. It was reported that

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diabetic patients have higher levels of glucose in their tears than the nondiabetics which may contribute to development of ocular infections.[7] The conjunctival flora in diabetic subjects may differ from that in nondiabetic subjects.

As such, the present study was designed to see the pattern of conjunctival bacterial flora in healthy individuals and diabetic patients.

Materials and Methods

The present study was a cross sectional study carried out in BSMMU during the period of January 2011 to December 2011.

Study population

Total 250 patients attending Eye OPD of BSMMU with complaints other than eye infections, mostly refractive error, were enrolled in the study. On the basis of history and glycemic status 50 patients were included in the diabetic group and another 200 were non-diabetics. Slit lamp examination was performed on each patient to find out any evidence of infection or inflammation.

Sample collection

With aseptic precaution and adequate light source, conjunctival swabs from both eyes were collected from all the patients. The swab was moistened with sterile normal saline and rubbed two to three times over the conjunctival sac from medial to lateral side.[8]

Microbiological methods

Smear was prepared with one swab from each sample and Gram staining was performed to demonstrate pus cells to exclude infection. Second swab was inoculated onto blood agar, chocolate agar, MacConkey agar, blood tellurite agar and Haemophilus selective agar media and were incubated at 37°C aerobically for 48 hours. Chocolate agar and Haemophilus selective agar plates were incubated in candle extinction jar. After 48 hours, all the organisms were identified by standard microbiological procedures namely colony morphology, Gram staining, pigment production and relevant biochemical tests (catalase, coagulase, novobiosin sensitivity, oxidase, MIU, mannitol fermentation, bile solubility, bile esculin test, rapid carbohydrate utilization test, growth factor requirement test, Haemophilus satellitism and butyrate esterase test).[9,10]

Table 1: *Culture results of conjunctival swabs of diabetic and non-diabetic individuals*

Study group	Total Number	Positive cultures n (%)	Unilateral culture positivity n (%)	Bilateral culture positivity n (%)
Diabetic cases	50	32 (64)	18 (36)	14 (28)
Non-diabetic cases	200	76 (38)	57 (28.5)	19 (9.5)

Statistical analysis

All data were collected in a predesigned data sheet and checked, edited and analyzed using SPSS (Statistical Package of Social Science) software. The data obtained from healthy individuals and diabetic patients were compared by Chi-square (χ^2) test.

Results

A total of 500 conjunctival swabs were studied from 250 participants. Out of total 500 conjunctival swabs, 400 were collected from both eyes of 200 non-diabetic patients and 100 from 50 diabetic patients. Significant number of culture was positive in diabetic patients (64.0%) compared to non-diabetics (38.0%). Table-1 shows that the rate of positive culture from both and single eyes was higher in diabetic patients (28% and 36% respectively) compared to the non-diabetic (9.5% and 28.5%).

Table 2: *Pattern of bacteria isolated from conjunctival swabs*

Bacterial species	Non-diabetic n (%)	Diabetic patients n (%)
S. epidermidis	62 (65.26)	19 (41.30)
S. aureus	4 (4.21)	7 (15.22)
S. saprophyticus	4 (4.21)	0 (0)
Viridans streptococci	6 (6.32)	4 (8.69)
S. pneumonia	0 (0)	1 (2.17)
Diphtheroids	2 (2.11)	0 (0)
Moraxella sp.	7 (7.37)	3 (6.52)
Pseudomonas sp.	7 (7.37)	3 (6.52)
H. influenza	3 (3.16)	2 (4.35)
E. coli	0 (0)	3 (6.52)
Enterobacter	0 (0)	4 (8.33)

Note: Total culture positive samples in non-diabetic patients were 95 (out of 400 swabs) and in diabetic patients were 46 (out of 100 swabs) irrespective of unilateral or bilateral positivity.

Among the culture positive samples, different bacterial species were isolated from conjunctival swabs of diabetic and non-diabetic patients. *S. epidermidis* was the most commonly isolated bacteria in both the study population but it was higher in non-diabetics (65.26%). Other isolated bacteria were *S. aureus*, S. *saprophyticus,* viridans *streptococci, Moraxella sp, H. influenzae* and *Pseudomonas sp* in different percentages among the both groups (Table-2). *S. saprophyticus* (4.21%) and *Diphtheroids* (2.11%) were isolated only in non-diabetic patients while *S. pneumoniae* (2.17%), *Esch. coli* (6.52%) and *Enterobacter* sp (8.3%) were isolated in diabetic patients only (Table-2).

Discussion

The conjunctival sac is parasitized with microflora that changes dynamically throughout the life time because of its long-term exposure to the environment and these flora are part of the defense mechanism of the eye in preventing colonization by more pathogenic microorganisms.[11]

Diabetes is associated with reduced immune function. Polymorphonuclear neutrophils in diabetic patients show alterations in chemotaxis, adherence, phagocytosis, intracellular killing, and bactericidal activity.[12] It contributes to more frequent isolation of organisms in diabetic individuals.

In the present study, conjunctival bacterial flora was isolated more frequently in diabetic patients (64%) than the non-diabetics (38%). Martin *et al.* (2004) also observed higher frequency of conjunctival culture positivity in diabetic group than those of nondiabetic group (94.18% vs. 73.33%).[13] In their study, they have correlated the frequency of culture positivity with diabetic retinopathy and the frequent bacterial isolation in those patients indicated that retinopathy might be a factor for altered conjunctival flora.

In the present study, *S. epidermidis* was isolated in highest percentage among all the isolates, both in non-diabetic (65.26%) and diabetic group (41.30%). Though this percentage is little higher in non-diabetic group, the lower isolation of *S. epidermidis* in diabetics is compensated by a higher isolation of *S aureus* (15.22%), viridans *streptococci* (8.69%), *E. coli* (6.52%) and *Enterobacter* (8.33%). It suggests that there is no definite cause of higher isolation rate of any specific bacteria in conjunctival swab of diabetic patients in comparison to non-diabetic patients. Overall colonization rate of bacterial flora in diabetic group was higher, because they had higher levels of glucose in their tears which may contribute to the development of ocular infections.[14] It may be mentioned that though anaerobic bacteria and fungi are an important part of conjunctival flora, they were not evaluated in the present study. There is significant change of conjunctival bacterial flora in diabetic patients in comparison to non-diabetic patients. *S. epidermidis* was the predominant organism in all study populations. The higher culture positivity rate in diabetic patients probably reflects a more permissive environment for bacterial growth in eyes of the diabetic patients.

References

1. Snell RS and Lemn MA. Conjunctiva. In: Clinical anatomy of eye. 2nd edition. Blackwell Science, USA. 1998; 108-112.
2. Tiffany JM. Tears in health and Disease. *Eye* 2003; **17**: 923–926.
3. Todar K. The normal bacterial flora of humans, Online Text Book of Bacteriology. 2009; Available at kentodar@textbookofbacteriology.net. Accessed on 2/01/2012.
4. Kowalski PR and Roat IM. Normal flora of the human conjunctiva. In: Tasman W and Jaeger EA eds. Duane's Ophthalmology. Lippincott William and Wilkins, Philadelphia 2006; Volume 2.
5. Therese KL & Madhavan HN. Microbiological procedures for diagnosis of ocular infections, L & T Microbiology Research Centre, Vision Research Foundation l8, College Road, Chennai 600 006. 2004.
6. Rich J and Lee JC. The pathogenesis of *Staphylococcus aureus* infection in the diabetic NOD mouse. *Diabetes* 2005; **54**: 2904-2910.
7. Skarbez K, Priestley Y, Hoepf M and Koevary SB. Comprehensive review of the effects of diabetes on ocular health. *Expert Rev Ophthalmology* 2010; **5**: 557–577.
8. Thomson BR and Bernard KA. Specimen Collection, Transport & Processing: Bacteriology. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfallier MA eds. Mannual of Clinical Microbiology. 9th edn. ASM Press, Washington D.C. 2007; 296.
9. Cheesbrough M. Microbiological tests. In: District Laboratory Practice in Tropical Countries. Cambridge University Press 2000; 64-404.

10. Isenberg HD. Biochemical tests for identification of aerobic bacteria. In: Clinical Microbiology Procedures Handbook. 2nd edition, ASM Press 2007; pp 3.17.7.
11. Liu J, Li J, Hu J and Xie H. Identification and quantitation of conjunctival aerobic bacterial flora from healthy residents at different ages in Southwest China. *African J Microbiol Res* 2011; **5**: 192-197.
12. Delamaire M, Maugendre D, Moreno MC *et al*. Impaired leucocyte functions in diabetic patients. *Diabet Med* 1997; **14**: 29-34.
13. Martins EN, Alvarenga LS, Höfling-Lima AL *et al*. Aerobic bacterial conjunctival flora in diabetic patients. *Cornea* 2004; **23**: 136-142.
14. Wood M. Conjunctivitis: Diagnosis and Management. *Comm Eye Health* 1999; **12**: 19-20.